

Attorney Docket No. CFBF-P01-021

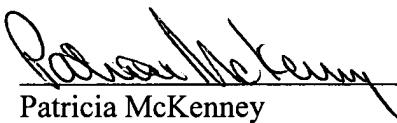


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Timothy A. Springer et al. Examiner: Maher M Haddad
Serial No.: 09/945,265 Art Unit: 1644
Filing Date: August 31, 2001
For: MODIFIED POLYPEPTIDES STABILIZED IN A DESIRED CONFORMATION AND METHODS FOR PRODUCING SAME

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Mail Stop AF, Commissioner for Patents, Box 1450, Alexandria, VA on 11/20/06.



Patricia McKenney

DECLARATION UNDER RULE '132

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Motomu Shimaoka , MD, PH.D, hereby declare and state as follows:

1. I am a research scientist at The CBR Institute for Biomedical Research, and I am a co-inventor and an applicant of the above-identified patent application. My curriculum vitae is attached hereto as an Exhibit.

2. I have read the Office Action of September 22, 2005, mailed by the US Patent and Trademark Office in this application ("Office Action"). I understand that paragraphs 3 and 6 of the Office Action raise certain questions regarding possible discrepancies between certain published sequences for the LFA-1 protein, and the corresponding wild type and modified amino acid sequences, as shown in SEQ ID NO:1 and SEQ ID NO:2 of the present application. In particular, the Office Action states that there are a discrepancy involving the identity of the amino acids at positions 284, 287, 289, 294, 301 of SEQ ID NO:2 and the published sequence.

3. In my opinion, there is no real discrepancy in the sequences noted in the Office Action for the following reasons. The sequences in the present application are numbered based on the mature LFA-1 protein. In contrast, the sequences of the publications referenced in the Office Action are numbered based on the LFA-1 precursor protein. The LFA-1 precursor protein contains 25 more residues than the mature protein. These additional residues comprise the signal peptide that is removed upon maturation. Thus, the K287 and K294 amino acid positions of the present invention correspond to amino acid positions K312 and K319, respectively, of the published sequences.

4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Date: 1/17/2006



Motomu Shimaoka (Title)

MD, PhD

CURRICULUM VITAE**Motomu Shimaoka, M.D., Ph.D.****DATE PREPARED: 12/21/05**

Name : Motomu Shimaoka

Office Address: 200 Longwood Ave., Rm 253, Boston, MA 02115

Home Address : 73 Longwood Ave. #2F, Brookline, MA 02446

email: shimaoka@cbrinstitute.org

Place of Birth: Nara, Japan

Visa status: U.S. Permanent Resident

Education :

| | | |
|------|----------------------|--|
| 1989 | M.D. | Osaka University Medical School, Osaka, Japan |
| 1997 | Ph.D. (Cell Biology) | Osaka University Graduate School, Osaka, Japan |

Postdoctoral Training

| | | |
|-----------|---------------------|---|
| 1989 | Clinical Fellow | Department of Anesthesiology, Osaka University Hospital, Osaka, Japan |
| 1989-1992 | Clinical Fellow | Department of Anesthesiology, Osaka Prefecture Hospital, Osaka, Japan |
| 1992-1993 | Clinical Fellow | Intensive Care Unit, Osaka University Hospital, Osaka, Japan |
| 1993-1995 | Postgraduate Fellow | Department of Anesthesiology, Osaka University Medical School, Osaka, Japan |
| 1993-1994 | Research Associate | Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan |
| 1995-1998 | Research Associate | Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan |
| 1998-2000 | Research Fellow | Center for Blood Research, Harvard Medical School, Boston, MA |

Licensure and Certification:

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|------|--|
| 1998 | Diplomat of Board of Japan Society of Anesthesiology |
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| 1991 | Registered Anesthetist |
| 1989 | Japanese Medical License Registration |

Academic Appointments:

| | | |
|-----------|---------------------|--|
| 1995-2001 | Assistant Professor | Department of Anesthesia, Osaka University Medical School, Osaka Japan |
| 2001-2003 | Instructor | Department of Anesthesia, Harvard Medical School, Boston, MA |
| 2003- | Assistant Professor | Department of Anesthesia, Harvard Medical School, Boston, MA |

Hospital or Affiliated institution Appointments:

| | | |
|--------------|---------------------|---|
| 1995-2001 | Staff Intensivist | Intensive Care Unit, Osaka University Hospital, Osaka, Japan |
| 2000-present | Junior Investigator | The CBR Institute for Biomedical Research, Harvard Medical School, Boston, MA |

Major Visiting Appointments:

| | | |
|-----------|---------------------|--|
| 1995-1998 | Anesthetist | Toyonaka City Hospital, Osaka, Japan |
| 1995-1998 | Anesthetist | Tondabayashi City Hospital, Osaka, Japan |
| 2002- | Research consultant | Respiratory Care, Mass. General Hospital, Boston, MA |

Major Committee Assignments:

| | | | |
|-----------|-----------------------|--------|---|
| 1995-1998 | Infection Controlling | Member | Osaka University Hospital, Osaka, Japan |
|-----------|-----------------------|--------|---|

Professional Societies:

| | | |
|----------------|---|--------|
| 1995 – 1999 | Japanese Society for Immunology | member |
| 1995 – 1997 | American Society for Anesthesiologists | member |
| 1989 – 2004 | Japanese Society of Intensive Care Medicine | member |
| 1989 – present | Japanese Society of Anesthesiology | member |
| 2004 – present | American Society of Hematology | member |

Editorial Boards:

| | | |
|------|-----------------|----------------------------------|
| 1998 | Ad hoc reviewer | Clinical Infectious Disease |
| 2002 | Ad hoc reviewer | European Journal of Biochemistry |
| 2005 | Ad hoc member | NIH MOSS Study Section |

Awards and Honors:

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|------|---|
| 1997 | Anesthesia and Intensive Care Medicine, Osaka University Medical School, Research Award |
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Funding Information

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|--|------------------|
| NIH RO1 (R01-AI063421, Shimaoka) | 12/1/04-11/30/09 |
| PI: Motomu Shimaoka | |
| Title: Integrin LFA-1 antagonists | |
| The major goals of this project are to develop and characterize novel antagonists specific to the active conformation of integrin LFA-1. | |

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| NIH PO1 (HL48675, Springer) | 9/1/05-8/31/10 |
| PI: Motomu Shimaoka | |
| Title: The aberrant activation of integrins on leukocytes | |
| This is one of five projects in NHLBI's Program Project Grant, 'Integrins and modular surface proteins in vasculature. I serve as a project leader. The aim of this project is to study the aberrant activation of integrins <i>in vivo</i> by generating novel genetically-engineered mice. | |

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| American Society of Hematology Junior Faculty Scholar Award | 7/1/04-6/30/06 |
| PI: Motomu Shimaoka | |
| Title: Conformational regulation of integrin $\alpha_L\beta_2$ (LFA-1) | |

The major goals of this project are to understand the importance of the conformational regulation of integrin LFA-1 by using mutant mice that express the active conformation of LFA-1.

Regional, national, or international contributions

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| 2005 | Invited speaker, 2nd Annual Symposium in Cellular, Molecular and Clinical Research in Surgery, Boston |
| 2005 | Invited speaker, CBR Institute for Biomedical Research, Boston |
| 2005 | Invited speaker, Dept Anesthesia, Children's Hospital, Boston |
| 2004 | Invited speaker, Institute of Medical Science, the University of Tokyo, Japan |
| 2004 | Invited speaker, Department of Anesthesiology, Osaka University Medical School, Osaka, Japan |

- 2004 Invited speaker, Gastroenterology, Mass General Hospital, Boston, MA
- 2004 Invited speaker, The CBR Institute for Biomedical Research, , Boston, MA
- 2002 Invited speaker, Dyax Corp., Cambridge, MA
- 2002 Invited speaker, The Center for Blood Research, Harvard Meidcal School, Boston, MA
- 2001 Invited speaker, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
- 2001 Invited speaker, Department of Immunology, Osaka Medical Center for Maternal and Child Health, Osaka, Japan
- 1997 Invited speaker, Department of Anesthesiology & Critical Care Medicine, Osaka University Medical School, Osaka, Japan
- 1997 Invited speaker, The Center for Blood Research, Harvard Meidcal School, Boston, MA
- 1997 Invited speaker, Laboratory of Immunology and Vascular Biology, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.
- 1997 Invited speaker, Department of Anesthesiology, Osaka University Medical School, Osaka, Japan.
- 1997 Lecturer, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada
- 1997 Invited speaker, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada
- 1997 Session Chairman, The 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada
I chaired a workshop titled, “Nosocomial Pneumonia”
- 1996 Invited speaker, The 23rd Congress of Japanese Society for Intensive Care Medicine, Tokyo, Japan
- 1996 Invited speaker, The 16th Congress of Japanese Society for Clinical Anesthesia, Tokyo, Japan
- 1996 Lecturer, The 5th Meeting of Association for Prevention of Stomatological Infection, Kobe, Japan
- 1995 Lecturer, The 42nd Congress for Japanese Society of Anesthesiologists, Hamamatsu, Japan
- 1995-1998 Lecturer, Department of Anesthesiology & Critical Care Medicine, Osaka University Medical School, Osaka, Japan

1993 Lecturer, Japanese Society for Microbiology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Publications:**Original**

- 1) **Shimaoka, M.**, Yoh, M., Segawa, A., Takarada, Y., Yamamoto, K., and Honda, T. Development of enzyme-labeled oligonucleotide probe for detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* 1994. 32: 1866-1869.
- 2) **Shimaoka, M.**, Iida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda, T., and Yoshiya, I. NOC, a nitric-oxide-releasing compound, induces dose dependent apoptosis in macrophages. *Biochem. Biophys. Res. Commun.* 1995. 209: 519-526.
- 3) **Shimaoka, M.**, Yoh, M., Takarada, Y., Yamamoto, K., and Honda, T. Detection of the gene for toxic shock syndrome toxin 1 in *Staphylococcus aureus* using enzyme-labeled oligonucleotide probes. *J. Med. Microbiol.* 1996. 44: 215-218.
- 4) **Shimaoka, M.**, Iida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda, T., and Yoshiya, I. Ketamine inhibits nitric oxide production in activated macrophages. *Br. J. Anesthesia* 1996. 77: 238-242.
- 5) Okuda, Y., Sakoda, S., **Shimaoka, M.**, and Yanagihara, T. Nitric oxide induces apoptosis in mouse splenic T lymphocytes. *Immunol. Lett.* 1996. 52: 135-138.
- 6) **Shimaoka, M.**, Ikeda, M., Iida, T., Taenaka, N., Yoshiya, I., and Honda, T. Fucoidin, a potent inhibitor of leukocyte rolling, prevents neutrophil influx into phorbol-ester-induced inflammatory sites in rabbit lungs. *Am. J. Respir. Crit. Care Med.* 1996. 153: 307-311.
- 7) Sugimoto, M., **Shimaoka, M.**, Hagihira, S., Fujino, Y., Nishimura, S., Kamata, S., Taenaka, N., and Yoshiya, I. Tension pneumopericardium following tracheoplasty for congenital tracheal stenosis. *Anaesth. Intens. Care* 1997. 25: 539-541.
- 8) Yamada, K., **Shimaoka, M.**, Nagayama, K., Hiroi, T., Kiyono, H., and Honda, T. Bacterial invasion induced interleukin-7 receptor expression in colonic epithelial cell line, T84. *Eur. J. Immunol.* 1997. 27: 3456-3460.
- 9) **Shimaoka, M.**, Fujino, Y., Taenaka, N., Hiroi, T., Kiyono, H., and Yoshiya, I. High frequency oscillatory ventilation attenuates the activation of alveolar macrophage and neutrophil in lung injury. *Critical Care (Lond)* 1998. 2: 35-39.
- 10) Sugimoto, M., **Shimaoka, M.**, Hosotsubo, K., Tanigami, H., Taenaka, N., Kiyono, H., and Yoshiya, I. Upregulation of Fas ligand mRNA expression in peripheral blood mononuclear cells after major surgery. *Clin Exp Immunol* 1998. 112: 120-125.
- 11) Egawa, T., **Shimaoka, M.**, Shimizu, N., Hagihira, S., Fujino, Y., Nishimura, S., Taenaka, N., Yoshimine, T., Yoshiya, I. A case of multiple aneurysms of the vein of Galen with heart failure due to persistent fetal circulation. *J. Anesthesia* 1998 12: 100-102.
- 12) Izumi, R., **Shimaoka, M.**, Nagaoka, C., Komaki, M., Mizutani, A., Yoh, M., Honda, T., Taenaka, N., Yoshiya, I. Effectiveness of hand-disinfection by a flow water system using electrolytic products of sodium chloride, compared with a conventional method using alcoholic solution in a intensive care unit (letter). *Critical Care (Lond)* 1998 2: 79-80.

- 13) Nishimura, S., Fujino, Y., **Shimaoka, M.**, Hagihiro, S., Taenaka, N., Yoshiya, I. Circadian secretion pattern of melatonin after major surgery. *J. Pineal. Res* 1998 25: 73-77.
- 14) Iijima, H., Takahashi, I., Hiroi, T., **Shimaoka, M.**, Kawano S., Nagano, K., Hori, M., Kiyono, H. Orally administered cholera toxin prevents murine intestinal T cell from staphylococcal enterotoxin B-induced anergy. *Gastroenterology* 1998, 115: 1197-1204.
- 15) **Shimaoka, M.**, Hosotsubo, K., Sugimoto, M., Sakaue, G., Taenaka, N., Yoshiya, I., Kiyono, H. Influence of surgical stress on T cells: enhancement of early phase of lymphocytes activation. *Anesth Analg* 1998 87: 1431-1435.
- 16) Sugimoto, M., **Shimaoka, M.**, Taenaka, N., Kiyono, H., and Yoshiya, I. Satellate ganglion block attenuates lymphocyte activation. *Reg Anesth Pain Med* 1999 24: 30-35.
- 17) Shimazu, T., **Shimaoka, M.**, Sugimoto, H., Taenaka, N., Hasegawa, T., & the Osaka HUS Critical Care Study Group. Does blood type B protect against haemolytic uraemic syndrome? an analysis of the 1996 Sakai outbreak of *Escherichia coli* O157:H7 (VTEC O157) infection. *J Infect* 2000 41: 45-49.
- 18) **Shimaoka, M.**, Shifman, J.M., Jing, H., Takagi, J., Mayo, S.L., Springer, T.A. Computational design of an integrin I domain stabilized in the open high affinity conformation. *Nature Struct. Biol.* 2000. 7: 674-678.
- 19) Imanaka, H., **Shimaoka, M.**, Matsuura, N., Nishimura, M., Ohta, N., Taenaka, N., Kiyono, H. Ventilator-induced lung injury is associated with neutrophil infiltration, macrophage activation and TGF- β mRNA upregulation in rat lung. *Anesth. Analg.* 2001. 92: 428-436.
- 20) Lu, C., **Shimaoka, M.**, Ferzly, M., Oxvig, C., Takagi, J., Springer, T.A. An isolated, surface-expressed I domain of the integrin α L β 2 is sufficient for strong adhesive function when locked in the open conformation with a disulfide bond. *Proc. Natl. Acad. Sci. U.S.A.* 2001 98:2387-2392
- 21) Lu, C., **Shimaoka, M.**, Zhang, Q., Takagi, J., Springer, T.A. Locking in alternative conformations of the integrin α L β 2 I domain with disulfide bonds reveals functional relationships among integrin domains. *Proc. Natl. Acad. Sci. U.S.A.* 2001 98:2393-2398
- 22) **Shimaoka, M.**, Lu, C., Palframan, R., von Andrian, U., Takagi, J., Springer, T.A. Reversibly locking a protein fold in an active conformation with a disulfide bond: integrin α L I-domains with high affinity and antagonist activity in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 2001 98:6009-6014.
- 23) Jun, C.D., **Shimaoka, M.**, Carman C.V., Takagi, J., Springer T.A. Dimerization and the effectiveness of ICAM-1 in mediating LFA-1-dependent adhesion. *Proc. Natl. Acad. Sci. U.S.A.* 2001 98: 6830-6835.
- 24) Ohta, N., **Shimaoka, M.**, Imanaka, H., Nishimura, M., Taenaka, N., Kiyono, H. Yoshiya, I. Glucocorticoid suppresses neutrophil activation in ventilator-induced lung injury. *Crit. Care Med.* 2001 29:1012-1016.
- 25) Sakaue, G., **Shimaoka, M.**, Fukuoka, T., Hiroi, T., Inoue, T., Hashimoto, N., Sakaguchi, T., Sawa, Y., Morishita, R., Kiyono, H., Noguchi, K., Mashimo, T. NF- κ B decoy

- suppresses cytokine expression and thermal hyperalgesia in a rat neuropathic pain model. *Neuroreport*. 2001. 12:2079-2084.
- 26) Jun, C.D., Carman, C.V., Redick, S.D., **Shimaoka, M.**, Erickson, H.P., Springer, T.A. Ultrastructure and function of dimeric, soluble intercellular adhesion molecule-1 (ICAM-1). *J. Biol. Chem.* 2001. 276:29019-29027.
 - 27) Ma, Q., **Shimaoka, M.**, Lu, C., Jing, H., Carman, C.V., Springer, T.A. Activation induced conformational changes in the I-domain region of LFA-1. *J Biol Chem* 2002. 277: 10638-10641.
 - 28) Takeuchi, M., Goddon, S., Dolnikoff, M., **Shimaoka, M.**, Hess, D., Amato M.B.P., Kacmarek, R.M. Set positive end-expiratory pressure during protective ventilation affects lung injury. *Anesthesiology* 2002. 97: 682-92.
 - 29) Salas, A., **Shimaoka, M.**, Carman, C.V., Chen S., Springer T.A. Transition from rolling to firm adhesion is regulated by the conformation of the I domain of the integrin LFA-1. *J. Biol Chem* 2002. 277: 50255-50262.
 - 30) **Shimaoka, M.**, Lu, C., Salas, A., Xiao, T., Takagi, J., Springer T.A. Stabilizing the α M inserted domain in alternative conformations with a range of engineered disulfide bonds. *Proc. Natl. Acad. Sci. U.S.A.* 2002. 99: 16737-16741.
 - 31) **Shimaoka, M.**, Xiao,T., Liu,JH, Yang Y., Dong,Y., Jun,CD., McCormack, A., Zhang,R., Joachimiak,A., Takagi,J., Wang,JH., Springer, TA. Structures of the α L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell*. 2003. 112: 99-111.
 - 32) Vorup-Jensen, T., Ostermeier, C., **Shimaoka, M.**, Hommel, U., Springer, T.A. Structure and allosteric regulation of the α X β 2 integrin I domain. *Proc. Natl. Acad. Sci. U.S.A.* 2003 100: 1873-1878.
 - 33) **Shimaoka, M***., Salas, A., Yang, W., Weitz-Schmidt, G., Springer, TA*. Small molecule integrin antagonists that bind to the β 2 subunit I-like domain and activate signals in one direction and block them in the other. *Immunity* 2003 19:391-402. (*Corresponding)
 - 34) Sedeek, K.A., Takeuchi, M., Suchodolski, K., Vargas, S.O., **Shimaoka, M.**, Schnitzer, J.J., Kacmarek, R.M. Open-lung protective ventilation with pressure control ventilation, high-frequency oscillation, and intratracheal pulmonary ventilation results in similar gas exchange, hemodynamics, and lung mechanics. *Anesthesiology* 2003. 99: 1102-1111.
 - 35) Yang, W., **Shimaoka, M.**, Chen JF., Springer, TA. Activation of integrin β -subunit I-like domains by one-turn C-terminal α -helix deletions. *Proc. Natl. Acad. Sci. U.S.A.* 2004. 101: 2333-2338.
 - 36) Yang, W., **Shimaoka, M.**, Salas, A., Takagi, J., Springer, TA. Intersubunit signal transmission in integrins by a receptor-like interaction with a pull spring. *Proc. Natl. Acad. Sci. U.S.A.* 2004. 101: 2906-2911.
 - 37) Salas A, **Shimaoka M**, Kogan AN, Harwood C, von Andrian UH, Springer TA. 2004. Rolling adhesion through an extended conformation of integrin α L β 2 and relation to α I and β I-like domain interaction. *Immunity* 2004. 20: 393-406.

- 38) Lu, C., **Shimaoka, M.**, Sales, A., Springer, T.A. The mechanism of action and binding sites for competitive, antagonistic, allosteric and agonistic antibodies to the I domain of integrin LFA-1. *J Immunol.* 173: 3972-8. 2004.
- 39) Xie, C., **Shimaoka, M.**, Xiao, T., Schwab, P., Klickstein, L.B., Springer, T.A., The integrin a subunit leg extends at a Ca²⁺-dependent epitope in the thigh/genu interface upon activation. *Proc. Natl. Acad. Sci. U.S.A.* 101: 15422-7. 2004.
- 40) Vorup-Jensen, T., Carman, C.V., **Shimaoka, M.**, Schuck, P., Svitel, J., Springer, T.A. Exposure of acidic residues as a danger signal for recognition of fibrinogen and other macromolecules by integrin $\alpha_x\beta_2$. *Proc. Natl. Acad. Sci. U.S.A.* 2005. 102:1614-9.
- 41) Song, G., Yang, Y., Liu, J-h., Casasnovas, J.M., **Shimaoka, M.**, Springer, T.A., Wang, J-h. Structural basis of ICAM recognition revealed in a complex between the binding domains of ICAM-3 and integrin $\alpha_L\beta_2$ at 1.65 Å. *Proc. Natl. Acad. Sci. U.S.A.* 102: 3366-71, 2005.
- 42) Zhang, M., Alicot, EM., Chiu, I., Li, J., Verna, N., Vorup-Jensen, T., Kessler, B., **Shimaoka, M.**, Chan, R., Friend, D., Mahmood, U., Weissleder, R., Moore, FD., Carroll, MC. Identification of the target self-antigens in reperfusion injury. *J. Exp. Med.* 2006 (in press)
- 43) Song, G., Lazar, GA., Kortemme, T., **Shimaoka, M.**, Desjarlais, JR., Baker, D., Springer, T.A. Rational design of ICAM-1 variants for antagonizing integrin LFA-1-dependent adhesion. *J. Biol. Chem.* 2006 (in press)

Reviews

- 1) **Shimaoka, M.**, Takagi, J., Springer, T.A. Conformational regulation of integrin structure and function. *Annu. Rev. Biophys. Biomol. Struct.* 2002. 31: 485-516.
- 2) **Shimaoka, M.**, Springer, T.A. Therapeutic antagonists and the conformational regulation of integrin structure and function. *Nature Rev Drug Discover* 2003. 2: 703-716.
- 3) **Shimaoka, M.**, Springer, T.A. Therapeutic antagonists and the conformational regulation of $\beta 2$ integrin. *Curr Topic Med Chem* 2004. 4: 1485-1495.

Educational Materials

Course syllabus

- 1) **Shimaoka, M.** Integrins-cell adhesion molecules, In: Diamandopoulos, GT, organizer. An advanced biomedical science program course PA514.0, Harvard Medical School; 2001. p221-224.